Overview of the study

Another five birth cohort study, the FinnTwin12 study, was started in September 1994, to examine genetic and environmental determinants of precursors of health-related behaviours, with particular focus on use and abuse of alcohol, in initially 11-12 year old twins. Major funding was obtained from the National Institutes of Health, USA. This research is cast within the perspective of developmental genetic epidemiology, asking whether precursors of risk behaviors are evident to parents, teachers, and classroom peers as early as age 12.

The study has a two-stage sampling design. The larger, first-stage study is an epidemiological investigation of five consecutive and complete birth cohorts of Finnish twin children, including questionnaire assessments of both twins and parents at baseline, starting with a family questionnaire (returned by 2,724 families, 87% participation rate) that was mailed late in the year before the twins reach age 12, with follow-up of all twins at age 14 and, in recently initiated data collection, again at age 17½ years. For the epidemiological study of Stage-1, we excluded families in which one or both co-twins were deceased or living outside Finland, families in which both co-twins lived apart from both biological parents, and families in which the Population Register Center contained no residential address for a twin.

First stage

Five separate questionnaires were mailed at baseline to each twin family: a family questionnaire, usually completed by the twins' mothers, which when returned, was immediately followed by a postal mailing of individual questionnaires to both parents and the two co-twins. Parents not resident with their twins were also contacted. The family questionnaire requested basic information on the twins' gestation and delivery and early development, their zygosity, and the specific school in which they were then enrolled. The baseline questionnaire assessment of the twins' parents includes an 11-item diagnostic screen for alcohol-related problems, enabling us to identify twin children at elevated risk of substance abuse. The twins' questionnaire included items on self-reported height & weight; time spent with twin and friends, time spent on different activities and hobbies, relationship with parents, atmosphere and caring at home; life satisfaction and pubertal development. At age 12, several months after the baseline questionnaires had been returned, we also sought a rating from parents and classroom teachers of all twins in the epidemiological sample, using a Multidimensional Peer Nomination Inventory (MPNI) (Pulkkinen, Kaprio, & Rose, 1999). Ratings were completed by 93% of teachers, and 92% of parents of the entire Stage-1 twin sample.

At age 14, most of the items asked of the twins at age 11-12 were repeated. In addition, items on frequency of use of alcohol and intoxication, use by peers of alcohol, own smoking status and peer smoking, alcohol expectancies, self-esteem, and peer use of drugs were included. The age 14 follow-up assessment of all twins, by postal questionnaire, is complete with a participation rate of 88%; 4740 questionnaires were returned out of 5362 mailed. The response rates were 87% for boys, and 90% for girls.

A second follow-up questionnaire, at age 17½ years, was initiated in autumn of 2000 and to be completed in the spring of 2005. Each cohort was mailed questionnaires in either March or April, or September-October closest to the time when they were aged 17.5 years. Again, many items are repeated, with additional age-specific items on health behaviors. We also ask for mobile phone and internet use, as new means of social interaction common among today's youth. So far, 3890 questionnaires have been returned out of 4212 mailed, a response rate of 92.4% for those already participating in earlier questionnaires.

As young adults, those not taking part in the second stage study (see below) have received a questionnaire representing the wave 4 follow-up of these twins.

This epidemiological first-stage of our FinnTwin12 study thus includes some 5,600 twins, and 5,000 of their biological parents, and, as expected from population-based ascertainment, the twins form equal proportions of brother-brother, sister-sister, and brother-sister pairs, permitting robust testing of gender modulation of genetic and environmental risks in the development of health behaviors, and appraisal of the magnitude and persistence of effects of variation in pubertal timing.
Second stage

Nested within this epidemiological, population-based study, is the second-stage of FinnTwin12, an intensive assessment of a sub-sample of twin families. Most of the sub-sample is selected at random, but this random sample is then enriched with twins at elevated familial risk for alcoholism. For inclusion in the intensively-studied sub-sample of Stage-2, we further required that (one or both of) the twins' parents returned the family questionnaire and gave us written permission to initiate school contact, that the family questionnaire contained no new information about the twins (e.g., studying abroad, living at home but severely handicapped) that made them ineligible for inclusion in the study; we then further required that both twins and at least one parent be Finnish-speaking, because it was cost-prohibitve to train and conduct structured psychiatric interviews in Swedish, as well as Finnish, language. As about six percent of the Finnish population speak Swedish as their mother tongue, all questionnaires were made in both languages. We have previously assessed the non-responders at each stage, and found no evidence for selection for family type (both vs. single parents), parental age, area of residence, type or sex of twin (Kaprio et al., 2002b).

Of families for whom permission was obtained for school contact, 1,035 were selected for intensive (Stage-2) study. About half are families in which the twins are assumed to be at elevated risk for alcohol problems, given the elevated scores on the Malmö-modified Michigan Alcoholism Screening Test (Mm-MAST) (Kaprio et al., 2002a), self-reported by one or both of their biological parents at baseline. We created an 11-item lifetime version of the Mm-MAST, adding two additional items to increase the predictive validity of the Mm-MAST as a screen for DSM-III-R/IV diagnoses of alcohol abuse and dependency. We have also found that the Mm-MAST is an effective screen for alcohol problems assessed by interview. Interviews of the parents in these families, using the Semi-Structured Assessment for Genetics of Alcoholism (SSAGA) instrument (Bucholz et al., 1994) have been completed, with a total of 1,864 interviews completed (participation rate ~ 90%).

In-school assessments of the twins, including peer nominations and individual behavioral testing, have been completed for all five cohorts. In this study, both co-twins were in the same class for nearly 90% of the twin pairs. The twins of the present study were in 1,002 classes, with a mean class size of 25 students. The twins had 23,200 classmates of the same age; 11,297 girls and 11,903 boys, for whom peer nominations were also obtained. These provide an extraordinary resource to compare the different aspects of the behaviours of twins and singletons in an exceptionally large and representative study population (Pulkkinen, Vaalamo, Hietala, Kaprio, & Rose, 2003), and we have shown that there are at most only minor differences in behaviour of twins and singletons. This indicates that twinship is often an advantage and among pre-adolescent twins does not seem to be a disadvantage compared to same-aged singletons.

Adolescent assessment at age 14

The adolescent SSAGA interviews are complete, yielding 1,854 completed interviews (90%). The twins’ interviews (with C-SSAGA-A, which provides diagnoses and symptom counts of several psychiatric disorders, including alcohol abuse and dependence, drug abuse and dependence, conduct disorder, oppositional disorder, depression, anxiety, suicidal behavior, anorexia, and bulimia) are highly standardized for the adolescent twins’ age, given the very dynamic nature of alcohol use and risk-associated behaviors in mid-adolescence; accordingly, we completed 90% of adolescent interviews within six months of the 14th birthday. We found no systematic source of bias in the small subset of parents and twins whom we have not been able to interview. Adolescent interviews are conducted face-to-face using custom software and a notebook PC to enter the twin’s responses online. In addition neuropsychological tests (Trails, Mazes, California Stroop) are conducted, and for the 1986 and 1987 saliva samples for hormonal assays (cortisol, testosterone) have been collected (Eriksson, Kaprio, Pulkkinen, & Rose, 2005).

We have examined associations of testosterone (T) and alcohol use in adolescent twin brothers, conducting both between- and within-family analyses. In analyses of twins as individuals, higher T levels, adjusted for time and season of sampling, characterized boys reporting ever drinking, more frequent intoxication, high density drinking, more alcohol symptoms, and diagnosed alcohol dependency on interview. Adjusting for pubertal development, only associations with symptom count and diagnosis remained significant. The association with frequent intoxication replicated among drinking-discordant twin brothers, effectively ruling out between-family confounds, but that association was not significant after adjustment for pubertal development (Eriksson et al., 2005).

Young adult assessment at mean age 22 (range 20-24)

The wave 4 data collection in 2006-2009 conducted structured psychiatric interviews at average age of 21.9 (SD 0.8,range 21-26) years, with neuropsychological assessments, and collection of DNA & serum samples (for basic biochemistry and metabolomics) from twins of 900+ families most informative for substance abuse/dependence with existing psychiatric interview data (at age 14 for the twins). During this one-day in-person assessment in Helsinki, we collected information about substance use, abuse & dependence (smoking, alcohol, cannabis and other drug use) based on questionnaires and interviews for DSM-IV diagnoses (alcohol, illicit drugs, depression, eating disorders, suicidal behavior and antisocial behaviour), but also about lifestyle (physical exercise, food habits), chemosensory preferences, mood and regulation of emotions (depression (GBI), mood (GHQ-12), personality (NEO-FFI), schizotypy (SPQ), sense of coherence (SOC)), general health (illnesses, body composition, metabolism, experienced health), cognitive functions (general cognitive capacity, spatial ability, memory, executive functioning, attentiveness, perceptual speed, motor dexterity, and social cognition as measured with ability to recognize emotional facial expressions), and work. The assessments were completed on 1347 twins (73% of target sample), of which 812 were seen in person (with neuropsychological testing) and the rest by telephone psychiatric interviews, mailed questionnaires and DNA collection (n=1285) by blood and/or saliva samples.

We now have genome-wide genotyping data (‘gwas’) from the wave 4 twins genotyped on the Illumina 670 custom chip. This has been imputed to Hapmap2 and 1000 Genomes. In addition 200+ metabolites, primarily of lipids and their fractions have been determined from all twins, who provided blood samples (n=788) (Kettunen et al, Nature Genetics 2012) and cotinine, 3-hydroxyccotinine and the nicotine metabolite ratio have been determined for smokers and their cotwins by professor Rachel Tyndale’s lab, University of Toronto for all occasional and daily smokers. We are currently completing the first epigenetic analyses using targeted gene-specific methylation assays, as well as genome-wide approaches (such as the Illumina Infinium 450k array). A small number of pairs have been invited to an intensive clinical assessment of metabolic health and obesity.

The FinnTwin12 dataset represents a very rich longitudinal design, with a genetically informative component (twins and parents).